

EXHIBIT A

14
PROJECT

Notebook No. _____

Continued From Page 13

- one plate of HUVEC P7 was trypsinized and 2×10^5 cells were plated per insert (8mm, coated with fibronectin), 29 inserts total. The media was changed every day until they reached complete confluency.

Neutrophil migration assay

Neutrophils migrate in response to a gradient of IL-8. IL-1 stimulated HUVEC cells will produce IL-8 at their cell surface where it presumably binds to heparin. By digesting heparin with our heparinase 3, the IL-8 will diffuse in media and neutrophils, who follow the gradient, will not be attracted to the cell surface, preventing them from migrating into the wells.

One well in which HUVEC cells were growing was stained with crystal violet (see protocol on page 6). The cells were confluent. Media was taken out of the wells and IL-1 (2 ng/ml) was added to 18 wells. The others received culture media only. After 4 hours, the wells were emptied again along with the inserts and Hep 3 at 1×10^6 ml in PBS was added to both the wells and the inserts, to 9 of them. The others received PBS only. The digestion was allowed to proceed for one hour at 37° . One insert that received Hep 3 was then stained to see if the cells had detached and it appeared that some had in fact lifted. A different coating is going to be tried next time.

All the inserts were emptied along with the wells and 0.2 ml of culture media was added to them. The wells and inserts that had some Hep 3 received culture media without heparin (BPM + 20% FS). 1.5×10^6 neutrophils were added to every insert and their migration was

Continued on Page 15

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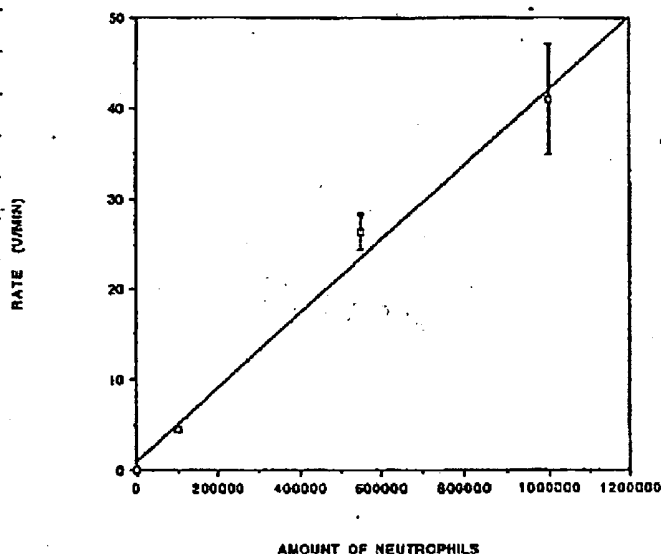
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Continued From Page 14

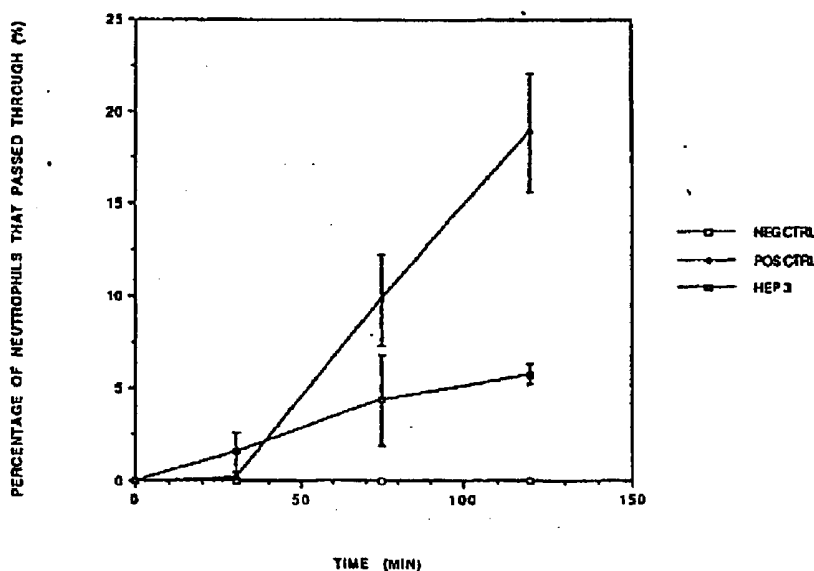
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stopped at 1/2 hr, 1 1/4 hr and 2 hours by taking out the inserts (3 filters of each series per time point). The bottom of the inserts were rinsed and the rinse was added to the content of the well. The samples were kept at -20°C O.N. and myeloperoxidase assay was done the day after.

MYELOPEROXIDASE ASSAY OF HUMAN NEUTROPHILS



MIGRATION OF NEUTROPHILS THROUGH A LAYER OF HUVEC CELLS DIGESTED WITH HEP 3 AT 1 IU/ML FOR AN HOUR



Raw Data
In Raw
Data Binder
p. 1 to 9

ued on Page 16

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